Seed Viability of Three *Salix* Species after 24 Months Storage at Two Moisture Contents and Four Temperatures

B.I. Daigle and J.D. Simpson
Natural Resources Canada, Canadian Forest Service - Atlantic Forestry Centre, National Tree Seed Centre, P.O. Box 4000, Fredericton, New Brunswick, E3B 5P7 Canada

Abstract

Seed of *Salix bebbiana*, *S. discolor*, and *S. eriocephala* was stored at two moisture contents (5.1-7.3% and 8.5-9.8%) and four temperatures (4, -20, -80, and −196°C) for 24 months. Seed stored at 4°C did not maintain viability. Storage at sub-freezing temperatures maintained seed viability for 24 months with little variation among treatments. Results demonstrate that cryopreservation of *Salix* seed is possible but does not offer any significant advantage over traditional storage at -20°C.

Introduction

The National Tree Seed Centre, created in 1967, is the only Seed Centre in Canada with a national mandate to obtain and store tree and shrub seed of native species for conservation and to provide seed for research purposes. Seed is collected and stored from across the geographic range of as many native species as possible. Almost all seed with orthodox storage requirements is stored in sealed containers at -20°C.

The *Salicaceae* family, which includes the genera *Salix* and *Populus*, comprises about 460 species worldwide, of which about 400 are willow species (Farrar, 1995). The three willow species used in this study: *Salix bebbiana*, *S. discolor*, and *S. eriocephala*, have a trans-Canada range and are all shrubs or shrubby trees ranging in height from 6–8 m. Willow and poplar seed is short-lived in nature and must germinate soon after dispersal. Wind-dispersed seed must find a suitable germination medium within a few days of release (Farrar, 1995; Young and Young, 1992). *Populus* and *Salix* seed will germinate while being transported by water and is ready to begin growing when it contacts soil (Farmer, 1997). Black willow (*S. nigra*) seed dispersed by water will remain viable for a longer period of time than if dispersed by wind (Pitcher and McKnight, 1990). Willow seed usually germinates within 12-24 hours upon landing on moist sand or alluvium (Young and Young, 1992).

Storage temperature and seed moisture content have long been known to affect the storage life of seed. Maroder *et al.* (2000) determined that seed moisture content of *S. alba* and *S. matsudana* seed was 40% immediately after release but quickly dropped to 11–12% within 3 hours under ambient conditions. Buch (1960) reported optimal storage moisture content for *Salix* and *Populus* seed to be between 4.0 and 7.5%. Tauer (1979) recommended drying the seed to a moisture content of 6–10%. Pence (1996) concluded that *P. deltoides* seed will survive storage in liquid nitrogen (LN) if the moisture content is below 15%. Seed of *Salicaceae*, consisting of an embryo and seed coat with almost no endosperm, does not store well unless frozen. Germination of *S. setchelliana* seed stored at room temperature in Petri dishes for 0, 5, 10, 15, and 20 days decreased from 100% at day 0 to 0% at day 20 (Douglas, 1995). Viability of *S. discolor* seed stored at 20°C decreased from 96 to 31% after 28 days, and seed of *S. bebbiana* dropped from 99 to 39% over the same period (Daigle and Simpson, 1998). *Populus balsamifera* seed stored at 7°C remained viable for only 8 months (Hellum, 1972). Viability of *S. alaxensis* seed stored at 3–5°C decreased from approximately 95% to below 10% after 10 months in storage, but the same seed stored at -10°C did not suffer any significant loss (Zasada and Densmore, 1977). Zasada and Densmore (1980) reported that willow and poplar seed could be successfully stored for up to 3 years at -10°C. Storage of *S. caprea* seed at -20°C provided better results than seed stored at -5 and 0°C (Simak, 1982). *Populus grandidentata* seed stored at -20°C at the National Tree Seed Centre germinated at 62% after 32 years. Although no record of cryopreservation of *Salix* seed could be found, cryopreservation of *Populus* seed has been successful. *Populus deltoides* stored in LN for 68 days germinated at the same level (100%) as seed stored at -20°C (Pence, 1996).

Seed storage objectives are primarily either short term, for forestry operations, or long term, for germplasm conservation (Bonner, 1990). Although the genus *Salix* is common in North America, there exist many instances where species are considered to be at risk. For example, *S. jejuna*, native to the province of Newfoundland, Canada, is classified as 'endangered' and *S. silicicola*, *S. brachycarpa* var. *psammophila*, and *S. turnorii*, indigenous to the northern part of the province of Saskatchewan, are classified as of 'special concern' (Committee on the Status of Endangered Wildlife in Canada, 2001). Black willow, a common species in the eastern United States, is at the northernmost extent of its range in New Brunswick, Canada, where it forms an

isolated population (Pitcher and McKnight, 1990). In Ohio, *P. balsamifera* and *P. heterophylla* are listed as threatened and potentially threatened, respectively (Pence, 1996).

The objective of this study was to evaluate the effect of two seed moisture contents and four storage temperatures on the viability of *Salix* seed. Seed at two moisture contents from *S. bebbiana*, *S. discolor*, and *S. eriocephala* was stored at 4, -20, -80, and -196°C. The results, after 2 years' storage, may serve as a guide for storage of seed collected from *Salix* spp. at risk.

Material and Methods

Seed from a single clone of *S. discolor* was collected on May 21, 1999 at Hamtown Corner, located just north of Fredericton, New Brunswick (46° 07' N; 66° 44' W). The catkins were immediately brought into the lab and laid on screen trays at 22°C to allow the capsules to dry and open. On May 25, after 4 days of drying, the partially opened catkins were placed in a rotating screen drum and subjected to warm airflow, which dislodged the seed from the cotton. The seed was sieved to remove larger plant material and lightly blown in an air aspirator to remove lighter particles. Percent moisture content (MC) was determined by drying two samples of seed in a forced draft oven for 16 hours at 103°C (ISTA, 1996) and MC calculated on a wet weight basis. The seed lot was halved and one of the sub-samples dried to a lower moisture content by air-drying in a forced draft oven at 30°C. The adjusted MC, or target moisture content (TMC), was calculated using the formula (IPGRI, 1996):

$$TMC = \frac{100 - (100 - IMC)(ISW)}{WDS}$$

Where: TMC = Target Moisture Content
IMC = Initial Moisture Content
ISW = Initial Seed Weight
WDS = Weight Dried Seed

Initial germination testing was carried out on May 26. The high and low moisture content sub-samples were further divided into storage samples weighing 0.35 g each; the seed was placed in 1.8-mL cryogenic vials and stored at 4°C on May 27 for 24 hours before being transferred to a -20°C freezer.

Seed of *S. bebbiana* and *S. eriocephala* was collected on May 28, 1999. The *S. eriocephala* seed was collected at the same location as the *S. discolor* and the *S. bebbiana* seed was collected at Mill Cove, New Brunswick (45° 53' N; 65° 00' W). The *S. bebbiana* seed was a bulk collection consisting of seed from four clones; the *S. eriocephala* catkins were collected from ten clones. The catkins were brought into the lab and laid on screen trays for 3 days at 22°C to allow the capsules to dry and open. The catkins were processed, their moisture content determined, and the lots divided into high and low moisture contents in the same manner described above. On June 1, a germination test was set up for these species and seed was placed in cold storage at 4°C for 24 hours, and then moved to frozen storage at -20°C. Samples of all three species that were to be stored at -80°C and in LN were removed from -20°C on June 11.

In addition to the initial germination test, seed was tested at 3, 6, 12, and 24 months. Two replicates of 100 seed each were used for the initial and 3-month tests, while four replicates of 100 seed each were used for the 6-, 12-, and 24-month tests. Vials of seed stored in LN and at -80°C were removed and placed in -20°C for 24 hours, and then these vials plus those stored at -20°C were placed at 4°C for 24 hours. Following this, all vials were left at room temperature for 4 hours before they were opened. The seed was then placed on moistened Kimpak™ in Petawawa germination boxes (Wang and Ackerman, 1983) and germinated for 10 days in Conviron™ G30 germination cabinets set at 20°C for 16 hours without light and 30°C for 8 hours with light and at a constant relative humidity of 85%. Germination was considered normal when the germinant had chlorophyll and was erect, the seed coat had shed, the cotyledons were open, and the hypocotyl hairs were capable of firmly anchoring the germinant on the substrate as described by Simak (1982).

Results

Moisture content of the seed after processing ranged from 8.5 to 9.8% and from 5.1 to 7.2% after drying. The greatest range in MC occurred with *S. discolor*, with the high and low MCs at 9.8 and 5.1%, respectively. The difference in MC for *S. bebbiana* and *S. eriocephala* ranged from 8.6 to 7.2% and 8.5 to 7.3%, respectively. Germination test results of the freshly collected seed (tested after processing and before drying) were 89.0% for *S. bebbiana*, 60.5% for *S. discolor*, and 71.5% for *S. eriocephala*.

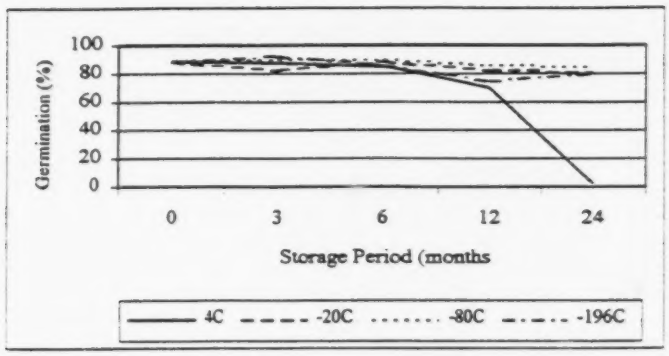

Figure 1. Germination (%) of *S. bebbiana* seed stored at four temperatures over a 24-month period.

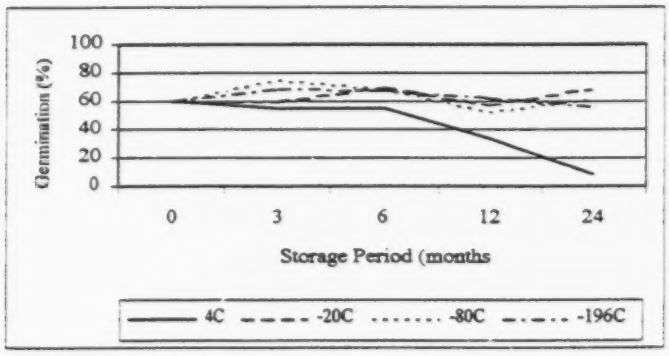

Figure 2. Germination (%) of *S. discolor* seed stored at four temperatures over a 24-month period.

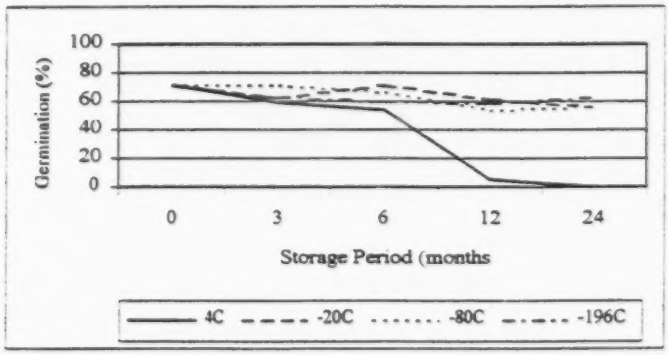

Figure 3. Germination (%) of *S. eriocephala* seed stored at four temperatures over a 24-month period.

Combining the results from each MC, little loss in viability was observed among storage temperatures up to 6 months. In fact, germination of *S. discolor* seed increased. However, after 6 months, seed viability of all three species stored at 4°C showed a marked decline (Figures 1, 2, and 3). The decline in germination over 24 months for seed in sub-zero storage averaged 7% for *S. bebbiana* and 13% for *S. eriocephala*, but results for *S. discolor* showed an increase of 1%.

After 24 months' storage, there was no significant difference in germination between MCs of *S. bebbiana* and *S. discolor* seed ($P < 0.0001$) and *S. eriocephala* seed ($P < 0.05$). Germination of seed for all species in frozen storage with a lower MC was always less than that for seed with a higher MC (Table 1). Germination differed

little among the three sub-zero storage temperatures within a given MC for each species and was only significantly different for *S. discolor* seed at 9.8% MC and *S. eriocephala* seed at 7.3% MC.

Table 1. Mean high-vigor germination of seed from three willow species stored at different moisture contents and four temperatures for 24 months.

	S. bebbiana Moisture Content		*S. discolor* Moisture Content		*S. eriocephala* Moisture Content	
Storage Temperature	8.6%	7.2%	9.8%	5.1%	8.5%	7.3%
4°C	2.0 a[1]	2.0 a	8.3 a	12.5 a	0.0 a	1.5 a
-20°C	81.5 b	79.5 b	68.3 b	55.8 b	56.0 b	43.8 bc
-80°C	84.5 b	72.3 b	61.0 bc	54.0 b	56.0 b	38.3 b
-196°C	80.3 b	79.3 b	56.0 c	53.3 b	62.0 b	52.0 c

[1]significant at $P < 0.0001$

Table 2. Mean abnormal germination of three species of willow seed stored at different moisture contents and four temperatures for 24 months.

	S. bebbiana Moisture Content		*S. discolor* Moisture Content		*S. eriocephala* Moisture Content	
Storage Temperature	8.6%	7.2%	9.8%	5.1%	8.5%	7.3%
4°C	3.8 a	5.0 a	4.8 a[1]	12.0 a	0.0 a[2]	0.8 a[2]
-20°C	5.8 a	6.0 a	12.3 b	15.5 a	16.8 b	18.5 b
-80°C	5.8 a	7.0 a	19.5 bc	15.3 a	19.3 b	28.8 c
-196°C	5.3 a	5.3 a	16.5 c	15.8 a	18.8 b	15.5 b

[1]significant at $P < 0.001$
[2]significant at $P < 0.0001$

Table 3. Proportion of abnormal germinants for seed of three willow species stored at different moisture contents and four temperatures for 24 months.

	S. bebbiana Moisture Content		*S. discolor* Moisture Content		*S. eriocephala* Moisture Content	
Storage Temperature	8.6%	7.2%	9.8%	5.1%	8.5%	7.3%
4°C	66.0	71.0	37.0	49.0	-	35.0
-20°C	7.0	7.0	15.0	22.0	22.0	30.0
-80°C	6.0	9.0	24.0	23.0	26.0	45.0
-196°C	6.0	6.0	23.0	23.0	23.0	23.0

There was no significant difference ($P < 0.0001$) in abnormal germination between the two MCs within each species. Abnormal germination was significantly different among the storage temperatures for *S. discolor* seed at 9.8% MC and *S. eriocephala* seed at both MCs (Table 2). Table 3 shows the abnormal germinants as a percentage of the total number of seed that germinated (normal + abnormal). The highest proportion of abnormal germinants occurred in *S. bebbiana* and *S. discolor* seed stored at 4°C. The proportion of abnormal germinants from seed in sub-zero storage was lowest in *S. bebbiana* and highest in *S. eriocephala*.

Discussion

The purpose of the experiment was to determine if *Salix* seed could be successfully stored at extremely low temperatures and what effect moisture content would have on the viability of the seed. Although it is well documented that poplar and willow seed can be successfully stored at sub-freezing temperatures (Zasada and Densmore, 1977; Tauer, 1979; Asakawa, 1982; Simak, 1982; Wang, 1982; Pence, 1996; Simpson and Daigle, 1998; Maroder *et al.*, 2000), the only documented storage temperatures below -20°C were for *P. deltoides* (Pence, 1996).

There exists ample evidence that *Salix* and *Populus* seed are extremely sensitive to storage at any temperature above freezing. In this experiment, storage at 4°C was clearly inferior. The results demonstrate that *Salix* seed

can be stored at temperatures below -20°C, however, no significant differences were found between any of the sub-freezing storage conditions, with the exception of the high MC of *S. discolor* and the low MC of *S. eriocephala* seed. These differences may be due to random variation in quality among the seed sub samples.

The literature suggests MCs between 5 and 15% are acceptable for storing seed of the *Salicaceae* family. Moisture content of seed used in this experiment ranged from 5.1 to 9.8%. Although not significantly different, seed with the lower MC was also less viable and tended to have more abnormal germinants. We do not believe that this is directly attributable to the decrease in MC of the seed. In order to lower the MC, the seed was placed in a forced draft oven at 30°C for 1 hour. This process may have injured the seed resulting in slightly less high-vigor germination and higher abnormal germination than observed at the higher MCs.

Time of collection has a profound effect on seed quality (Pence, 1996). Seed collected too early is of lower initial quality and does not store as well. Seed with low viability (e.g., stored seed) germinates more slowly and is often abnormal (von Wettstein, 1936). This was borne out by our findings. The seed stored at 4°C rapidly lost viability and had a higher proportion of abnormal germinants. The proportion of abnormal germinants was also high in *S. discolor* and *S. eriocephala* seed from frozen storage. In contrast, *S. bebbiana* seed had a low proportion of abnormal germinants. This may reflect the initial quality of the seed as *S. bebbiana* had the highest initial germination at 89%.

Based on these results, the following recommendations can be made:

1. *Salix* seed should be collected when the seed is ready to shed naturally. This ensures high quality seed with high germination and vigor that is able to withstand storage for a long period of time.
2. Storage at -20°C is sufficient. Although seed can survive storage at lower temperatures, no advantage is gained and the costs are higher.
3. Moisture content below 10% (fresh weight basis) should be used when storing seed at sub-zero temperatures. *Salix* seed loses moisture very quickly and lowering the MC to below 10% should not pose a problem. Moisture content should be lowered by air-drying or by placing seed in a desiccator. Avoid force-drying seed in an oven.
4. Seed should be processed and put in storage as soon as possible following collection. Willow seed deteriorates very quickly in nature and at room temperature. Also, when temporarily removing seed from storage for testing or distribution, the remaining seed should be returned to storage as soon as possible.
5. Seed should be stored in several small containers to mitigate the negative effects of taking the seed in and out of storage (i.e., for testing or distribution).

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